SOME CHARACTERISTICS OF B. CHAUVŒI

LEONARD W. GOSS,* RHEA E. BARBARIN, AND A. W. HAINES

From Research Laboratory, Parke, Davis and Company, Detroit, Mich.

INTRODUCTION

This paper deals with the methods of isolation of B. chauvœi from infected tissue and with its differentiation from the other anaerobes frequently found in blackleg and blackleg-like infections.

The organisms with which B. chauvœi is most frequently confused is Vibrion septique. The latter, as well as B. chauvœi, may at times be responsible for acute infections associated with emphysematous lesions, caused by gas-producing anaerobes in cattle. Both Vibrion septique and B. chauvœi are nonproteolyzers. This facilitates their differentiation from most other anaerobes in such infections.

A review of the literature on blackleg is superfluous as Heller [1] recently has covered the subject. A more concise summary of methods of differentiating anaerobes is to be found in the British Medical Research Committee Bulletins 12 and 39.

PREPARATION OF MEDIUMS

The mediums used in our study are the Hibler medium; 2% dextrose agar; liver broth; and liver agar.

Hibler Medium.—Beef liver, 500 gm., and brain, 500 gm., are ground; 1,000 c c of water added to the liver. Both are cooked in flowing steam one hour. The liver broth is strained through cheese cloth and cotton, 1% peptone and 0.5% salt added. The broth titrated to P_H 8.2. The medium is tubed; two parts of broth used to one part of brain. The tubes are autoclaved under 15 pounds' pressure for one hour.

Dextrose Agar.—One % peptone, 0.5% sodium chloride and 2% dextrose are added to beef infusion (beef, 500 gm., to water, 1,000 c c), titrated to P_H 8.2, 2% agar added and heated in flowing steam until the agar is completely dissolved. The medium is clarified with egg albumin, filtered, tubed and autoclaved under 15 pounds' pressure for 20 minutes.

Liver Broth.—Liver broth prepared like that used in Hibler medium is clarified with egg albumin, filtered, tubed and autoclaved under 15 pounds' pressure for 20 minutes.

Liver Agar.—Two % agar is added to liver broth, heated in flowing steam until the agar is completely dissolved (45 minutes), clarified with egg albumin, filtered, tubed and autoclaved under 15 pounds' pressure for 20 minutes.

Received for publication July 23, 1921.

* Department of Veterinary Pathology, Ohio State University, Columbus.

[1] Jour. Infect. Dis., 1920, 27, p. 385.

MORPHOLOGY OF B. CHAUVŒI

Fifteen strains of B. chauvœi were studied, 14 isolated from field cases of blackleg in Kansas, Michigan and Iowa, and one isolated from infected muscle received from the Bureau of Animal Industry in Washington. The morphology and cultural characteristics of these strains are as follows: The organisms occur singly or in pairs, very seldom in chains. They vary in size, but average 0.5 microns in width by 3 to 4 microns in length. They frequently contain granules, and become pleomorphic after incubation for from 24 to 48 hours. The most common variation in form is the clostridial or navicular. The organisms are sluggishly motile in young cultures but actively so in the exudate at the site of inoculation, in guinea-pigs dying from this infection.

Spores develop in from 18 to 24 hours in Hibler medium. They are elliptical, larger than the diameter of the bacterial cell, occupying a median or terminal position in the cell, giving it a lemon-like or pear-shaped appearance. Clostridial forms are found also in the fluid at the site of injection, after death. The morphology of B. chauvœi in the body of the guinea-pig differs from that of Vibrion septique. The latter frequently grows in long chains on the surface of the liver. This is not true of the former, as it grows singly or in pairs in this location. We have found B. chauvœi to be gram-positive in young cultures when stained by Stirling's modification of Gram stain. This is contrary to the assertions of Heller [1] and others. This variation may be due to the fact that these workers did not use the Stirling method. Old cultures become gram-negative.

CULTURAL CHARACTERISTICS

Dextose Agar.—Agar with 2% dextrose is useful in the differentiation of B. chauvœi from other anaerobes, and especially from Vibrion septique. The majority of the organisms found in emphysematous lesions grow very well in this medium, when planted in serial dilution in deep tubes. This is not true of B. chauvœi which fails to develop at all unless tissue or blood is carried over in the seeding. Even then the colonies formed are small and difficult to isolate. This fact is of great value in establishing the purity of B. chauvœi cultures.

Liver Medium.—When an extract in physiologic salt solution is made of fresh, sterile guinea-pig liver, and 2 c c are added to a tube containing 25 c c glucose agar, B. chauvœi colonies develop, while failing to do so in glucose agar without extract of liver. In the deep

tubes of beef liver agar described, a good growth is obtained. The colonies develop in from 18 to 24 hours. They are spherical or elliptical in shape, small and translucent. Vibrion septique, on the other hand, forms a fluffy colony in this medium.

When the agar concentration is lowered to 1%, B. chauvœi grows in colonies very similar in appearance to those of Vibrion septique in the 2% agar medium. B. chauvœi produces little or no gas in deep tubes of beef liver agar, after incubation for 24 hours. While cultures of Vibrion septique show early and extensive gas production, the agar being shattered in 24 hours.

When dilutions of a suspension of Vibrion septique are made in beef liver agar and poured into plates, which are then incubated in vacuo, good-sized colonies develop in 24 hours. B. chauvœi, on the other hand, fails to show growth, even after incubation for 3 days. This is a differential characteristic of great importance.

B. chauvœi ferments liver broth in fermentation tubes, with the formation of both acid and gas. Involution forms appear early, and spores develop rapidly in this medium. B. chauvœi will grow without the intervention of anaerobic conditions when placed in liver broth to which cubes of cooked liver have been added.

The most distinctive growth characteristic of this organism in liquid medium is its tendency to agglutinate. The medium remains cloudy for a short time during active growth, but clumping takes place rapidly, leaving the supernatant liquid layer as clear as if no growth had occurred.

Hibler Medium.—B. chauvœi grows under apparent aerobic conditions in this medium. Gas is produced; rapid agglutination occurs; the cultures have no odor. The brain tissue turns slightly pink, but is not digested. Neither B. chauvœi nor Vibrion septique give any evidence of proteoclastic activity in these mediums. The only odor is a faint indication of butyric acid. This is most easily detected when old cultures are placed on slides and treated slightly. Vibrion septique tends to grow in chains in Hibler medium. This is not true of B. chauvœi.

Litmus Milk.—Vibrion septique grows well when seeded in large amounts, by pipet, into litmus milk which has been freshly boiled to expel oxygen. An acid reaction occurs in 24 hours. No growth is obtained when inoculation is made by loop. In the case of B. chauvœi no growth results, even when 5 times the amount used for successful seeding of Vibrion septique is employed.

Gelatin.—In gelatin prepared with liver broth as a base, B. chauvœi forms fluffy colonies. The medium is liquified and gas produced by some strains of B. chauvœi, the colonies rapidly settling to the bottom of the tube.

Carbohydrate Fermentation

Beef infusion was inoculated with B. coli, incubated 24 hours at 37 C. to remove sugar. The colon organisms were removed by coagulation with egg white and 1% peptone and 2% carbohydrate added. The broth was then titrated to P_H 8.2, filled into fermentation tubes, autoclaved 20 minutes at 15 pounds' pressure, tested for sterility and then inoculated with 0.1 c c of a 48-hour Hibler culture. Readings were taken after incubation at 37 C. for 24 and 48 hours. Three lots of mediums were made at different times and 3 tubes inoculated with each organism. Table 1 shows the average results of these inoculations.

TABLE 1

Fermentation Tests

Carbohydrate	Malignant Edema (Novy)	B. Ghon-Sachs (Meyer)	Vibrion septique (Pasteur)	B. chauvœi (5 Strains Were Tested)	B. welchii 14	B. Botulinus 71
Glucose	A G	A G	A G	No growth	A G	A G
Saccharose	A	A	A	No growth	A G	A
Maltose	A G	A G	A G	No growth	A G	A G
Lactose	A	A G	A G	No growth	A G	A
Xylose	A	A	A	No growth	A G	A
Dulcite	A	A	A	No growth	A	A
Sorbite	A G	A	A	No growth	A	A G
Glycerin	A	A	A	No growth	No growth	A
Inulin	A	A	A	No growth	A G	A
Salicin	A	A G	A G	No growth	A	A
Mannite	A	A	A	No growth	A G	A
Liver broth	A G	A G	A G	A G	A G	A G
Levulose	A G	A G	A G	No growth	A G	A G
Dextrin	A	A	A	No growth	No growth	A
Raffinose	A	A	A	No growth	A	A
Amygdalin	A	A	A	No growth	No growth	A
Glycogen	A	A G	A G	No growth	A G	A

G, gas; A, acid.

Isolation of B. chauvœi from Infected Muscle

When suspected blackleg tissue is received it is examined microscopically. A small piece of the material is macerated in sterile salt solution, and a few c c of the suspension drawn into a capillary pipet and heated at 60 C. for 45 minutes.

These suspensions, heated and unheated portions, are seeded into liver agar and 2% glucose agar, serial dilutions being made. Hibler medium is also inoculated. Finally, a guinea-pig is injected subcutaneously with 1 c c of each suspension. Residual material is dried under aseptic precautions, for future use. This is done in vacuo at 37 C., or at atmospheric pressure in an oven at 55 C.

After incubation at 37 C. for 18-24 hours, the liver agar tube is examined for characteristic colonies. These are fished and planted

again in liver agar, glucose agar and Hibler medium. Great care must be exercised in picking the colonies, especially if anaerobes other than B. chauvœi are present.

These alternations should be repeated until purity is assured by morphologic study, failure to grow in 2% glucose agar, animal inoculations and protection tests. It is reiterated here that failure to grow in serial dilutions of deep tubes of glucose agar, along with characteristic growth of the same material in liver-agar, is a most important test for purity of the culture.

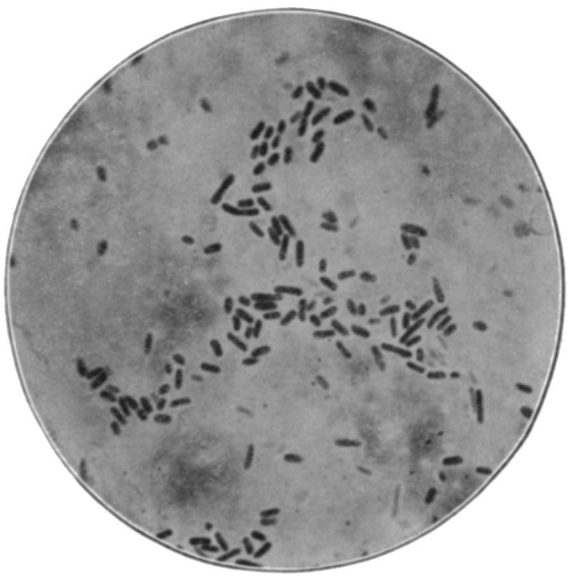

Fig. 1.—B. chauvœi from 16 hour Hibler culture, X 1,200; Gram stain, Stirling.

If the inoculated guinea-pig dies it should be examined promptly, gross lesions noted, smears made from the site of injection, and liver surface; and cultures made from the heart blood in liver agar, glucose agar and Hibler medium.

If the guinea-pig dies in less than 16 hours, death is probably due to a more invasive organism than B. chauvœi. For this reason B. chauvœi may easily be lost when it, in combination with Vibrion septique, is injected into a guinea-pig and cultures are made from the heart blood. For the purpose of separating blackleg from this organism guinea-pigs passively immunized with specific antitoxic serum may be used. By protecting the guinea-pigs against Vibrion septique and not B. chauvœi, death usually results from blackleg and the organism may be isolated in this way.

PATHOGENICITY OF B. CHAUVŒI

Guinea-Pigs.—The guinea-pig is the animal of choice in the experimental study of B. chauvœi. The cultures used in this investigation seem to vary considerably in virulence for these animals. At the same time, certain variations occurring in successive cultural and animal passages of one and the same strain, seriously weaken statements in regard to the variations in virulence between different strains.

Thus, strain 1, 24-hour Hibler culture, after passage through a guinea-pig, killed a 350 gm. animal in a dose of 0.02 c c. The same

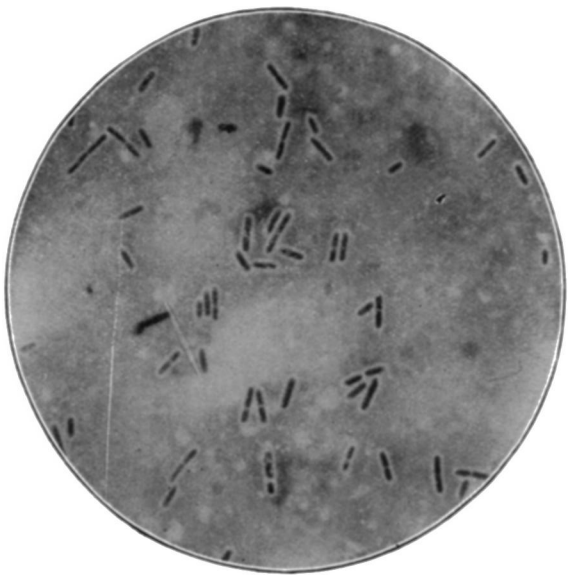

Fig. 2.—Impression from liver surface of guinea-pig injected with pure culture of B. chauvœi; Gram stain, Stirling; X 1,200.

strain, 24-hour Hibler culture, after this passage, required 0.2 c c to kill another guinea-pig of the same weight. These strange variations might be referred to differences in resistance of individual animals, uncontrollable variation in the same or different lots of culture medium. Or, again, organisms of markedly different virulence might occur side by side in a given strain, the more virulent type predominating at one time, the less virulent at another.

Vibrion septique produces more rapidly fatal infections in guinea-pigs than does B. chauvœi following the injection of like amounts of culture. The infections by the latter are characterized by a marked

swelling around the injection site some hours before death. When B. chauvœi infections are rapidly fatal, the blood at death may be devoid of organisms. If such animals are allowed to remain at room temperature for from one to two hours after death, the causative organisms appear on the liver surface and in the heart blood. On the other hand, Vibrion septique is of a more invasive character, organisms invariably being found in the heart blood at death.

The guinea-pigs in table 2 presented typical blackleg lesions. Those receiving 0.05 c c of culture did not die until the third day following injection. The hair over the lesions on these animals was easily removed. This is usually the case when the animals die slowly, and may occur in animals dying rapidly, due to the local tissue changes incident to the rapid bacterial multiplication beneath the skin.

TABLE 2

RELATIVE SUSCEPTIBILITY OF GUINEA-PIGS, WHITE MICE AND PIGEONS TO B. CHAUVŒI

Animal	Weight in Gm.	Amount Injected in c c	Result		
			24 Hours	48 Hours	72 Hours
Guinea-pig 1.....	350	0.1	Dead		
2.....		0.1	Dead		
3.....		0.05	Slight swelling	Marked swelling	Dead
4.....		0.05	Swelling	Marked swelling	Dead
5.....		0.025	Swelling	Dead	
White mouse 1.....	20	0.5	Dead		
2.....		0.5	Dead		
3.....		0.2	.....	Survived	
Pigeon 1.....	350	0.5	.....	Survived	

All injections made subcutaneously with B. chauvœi strain No. 3.

The entire abdominal and thoracic regions were hemorrhagic, certain areas of muscle being more deeply stained than others, especially in the axillary regions. There was a small amount of hemorrhagic fluid present and slight serogelatinous infiltration of the intramuscular tissue. A few small gas bubbles were present in the axillary spaces. No appreciable amount of gas was present in the intestines. The entire subcutaneous lesion brightened in color on exposure to air.

Bacteria at site of injection: Gram-positive bacilli in large numbers; many clostridia.

Bacteria on liver surface: Gram-positive bacilli appearing singly or in pairs, seldom in chains or filaments.

Pure culture of B. chauvœi was obtained from the heart blood.

White mice, according to von Hibler,[2] are refractory to B. chauvœi infection. We have found these animals to be susceptible to cultures highly virulent for guinea-pigs. However, mice are considerably more resistant, 2 or 3 times the dose fatal for guinea-pigs being required to produce infection in the first-mentioned animals.

After death there was slight subcutaneous edema at the point of inoculation.

Bacteria at site of inoculation: Gram-positive bacilli and clostridia.

Bacteria on liver surface: Gram-positive bacilli, singly and in pairs.

[2] Kolle de Wassermann, Handb. d. path. Mikroorg., 1912, 4, p. 788.

Pigeons, which are well known to be highly susceptible to B. welchii, are strongly refractory to B. chauvœi inoculations, according to von Hibler,[2] Arloing, Cornevin and Thomas.[3] The relative susceptibility of these animals is illustrated in table 2. Although the pigeon in table 2 did not die, we have been successful in producing fatal blackleg infections in pigeons. The animals in table 2 were injected simultaneously with the same culture.

The pectoral region was deeply hemorrhagic, friable and moist. The tissues were separated by tiny gas bubbles. The lesions resembled blackleg lesions in cattle having the sweet sickish odor characteristic of blackleg lesions in cattle.

Bacteria at site of inoculation: Gram-positive rods; clostridia.

Bacteria on liver surface: Gram-positive rods single and in pairs. Pure culture of B. chauvœi from heart blood.

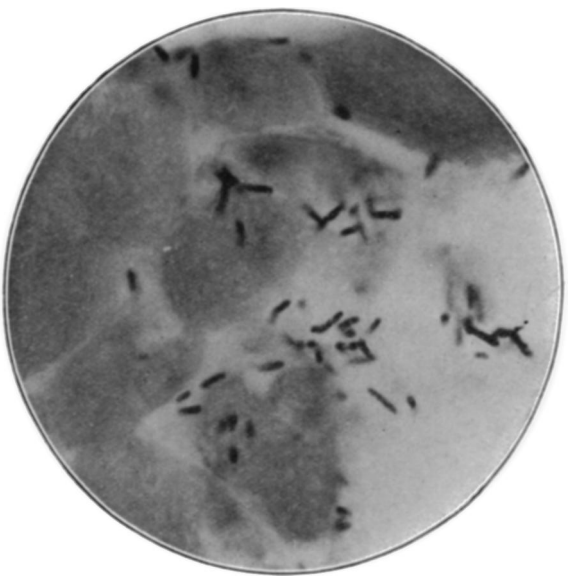

Fig. 3.—Section of calf muscle showing B. chauvœi; Gram stain, X 1,200.

Frequent mention is made in the literature regarding blackleg in sheep. Von Hibler [2] is of this opinion. Kitt [4] states, "In sheep whose caudal skin is not so close to the vertebra as in cattle, but has a substratum of spongy cell tissue, the inoculation into the tail apex produces a marked swelling and general infection; by application of an ice-bag, however, the local reaction can be prevented."

On the other hand, it is an interesting fact that we have never isolated true B. chauvœi from the muscle of sheep alleged to have

[3] Le charbon Symptomatique du boef, 1887, p. 87.

[4] Kolle u. Wassermann, Handbuch. d. path. Mikroorg., 1912, 4, p. 821.

died from a field case of blackleg. Anaerobes pathogenic for guinea-pigs were isolated, but invariably they were varieties other than the typical B. chauvœi. It was, therefore, decided to attempt the experimental infection of sheep with true B. chauvœi cultures. For this purpose cultures highly virulent for guinea-pigs were used.

Table 3 shows the results of intramuscular inoculation of B. chauvœi 15 into a lamb, a kid and a sheep. A Hibler culture grown 24 hours at 37 C. and placed in the refrigerator for two weeks was used. The lamb and sheep developed slight swellings at the point of injection and were lame. The kid succumbed to the infection.

TABLE 3

INOCULATION OF SHEEP, LAMB AND KID

Animal	Culture	Age of Culture, Weeks	Dose in c c	Results			
				18 Hours	24 Hours	28 Hours	96 Hours
Guinea-pig.....	15	2	0.1	Sick	Sick	Dead	
Kid.....	15	2	2.0	Leg swollen	Sick	Dead	
Lamb.....	15	2	2.0	Leg swollen	Sick	Lame	Recove e l
Sheep.....	15	2	2.0	Leg swollen	Sick	Lame	Recovere l

TABLE 4

INOCULATION OF SHEEP

Animal	Culture	Age of Culture	Dose in c c	Results		
				18 Hours	24 Hours	48 Hours
Guinea-pig.....	09	1 month	0.05	Sick	Sick	Dead
Sheep.....	09	1 month	5.0	Sick	Dead	
Guinea-pig.....	11	2 days	0.05	Sick	Sick	Dead
Sheep.....	11	2 days	5.0	Sick	Sick	Dead
Guinea-pig.....	13	5 months	0.02	Sick	Dead	
Sheep.....	13	5 months	0.50	Sick	Dead	
Guinea-pig.....	15	1 month	0.02	Sick	Dead	
Sheep.....	15	1 month	5.0	Sick	Dead	
Guinea-pig.....	16	2 months	0.02	Sick	Dead	
Sheep.....	16	2 months	5.0	Sick	Dead	
Guinea-pig.....	M	1 month	0.1	Dead		
Sheep.....	M	1 month	5.0	Dead		

Inoculations were made in the hind leg.

The results given in table 4 show that young, healthy sheep can be infected with B. chauvœi by injecting large doses of virulent culture.

One sheep in table 4 was injected with a 24-hour Hibler culture of an organism (M) sent to us as a culture of B. chauvœi isolated from a sheep supposedly dead of blackleg. The organism did not resemble our strains of B. chauvœi culturally. It did, however, resemble Vibrion septique culturally and in its pathogenicity for small animals.

Kid.—Serohemorrhagic edema; no gas in tissues; small amount of gas in the intestines; gram-positive bacilli single and in pairs on surface of the liver; pure culture of B. chauvœi from the heart blood.

Sheep.—All animals showed lesions similar to those found in cattle dying from blackleg infection; bloody exudate from nostrils and swelling of affected leg; muscle dark reddish black with characteristic blackleg odor; considerable congestion throughout abdominal cavity; liver light yellow in color; spleens enlarged and softened. Pure cultures of B. chauvœi were obtained from the heart blood of all the sheep injected with strains of B. chauvœi.

The sheep injected with culture M. died 5 hours earlier than the sheep injected with B. chauvœi. The clinical symptoms and lesions were similar to those of blackleg, but the organism isolated from the heart blood was a Vibrion septique group organism which grew luxuriantly in glucose agar.

It would appear from tables 3 and 4 that while sheep are somewhat refractory to injections of B. chauvœi, fatal infections may be produced with large doses of highly virulent cultures. Field cases of blackleg in sheep are rarely found in comparison to the frequency with which it occurs in cattle. This undoubtedly is due to the relative insusceptibility of sheep to natural infection with B. chauvœi.

In common with previous observers, it has been found that rabbits are highly refractory to large doses of virulent B. chauvœi. White rats are also resistant to such cultures.

IMMUNOLOGIC TESTS

Protective Power of Antiblackleg Serum (Horse) Against B. Chauvœi and Vibrion Septique.—While data of value in differentiating B. chauvœi from Vibrion septique have been obtained, as presented in the foregoing pages, it was considered desirable to discover a specific immunity reaction as an ultimate means of differentiation. Roux and Chamberland [5] state that animals vaccinated against blackleg resist Vibrion septique infections. Their opinion is shared by Duenschmann [6] who claims that antiblackleg serum protects against Vibrion septique infections. On the other hand, Leclainche and Vallée,[7] supported by immunity experiments, insist that there is a clear-cut difference between these organisms. The results about to be presented bear out the latter opinion.

Guinea-pigs were injected subcutaneously with 0.1 c c of antiblackleg serum. This serum was obtained from horses hyperimmunized with virulent cultures of B. chauvœi. Twenty-four hours after the injection of this serum, 2 of the guinea-pigs were injected with large

[5] Ann. de l'Inst. Pasteur, 1887, 1, p. 561.
[6] Ibid., 1894, 8, p. 402.
[7] Ibid., 1900, 14, p. 590.

amounts of virulent culture of B. chauvœi 8. Two others were injected with a virulent culture of Vibrion septique (Pasteur). Controls, injected with normal horse serum, received varying doses of the same cultures. The inoculations were made subcutaneously with 24-hour Hibler cultures. The results of this experiment are striking and are summarized in table 5.

Animals 1-4 and 12-14 showed typical blackleg lesions.

Reddish fluid oozed through the skin of animals 8-11. The subcutaneous and intramuscular tissues were slightly hemorrhagic, containing a serohemorrhagic fluid and small gas bubbles. The tissues were soft and jelly-like in appearance. The intestines were covered with small hemorrhages, considerable gas being present. The heart blood was partially coagulated.

Site of Injection: Gram-positive bacilli, chains, filaments and clostridia.

Liver Surface: Gram-positive bacilli, chains and filaments.

TABLE 5

PROTECTIVE POWER OF ANTIBLACKLEG SERUM AGAINST B. CHAUVŒI; FAILURE TO PROTECT AGAINST VIBRION SEPTIQUE

Guinea-Pig	Serum		B. chauvœi 8	Vibrion septique (Pasteur)	Result		
	Anti-Blackleg	Normal Horse Serum			24 Hours	48 Hours	96 Hours
1	—	—	0.02	—	Slight swelling	Dead	
2	—	—	0.05	—	Swelling	Dead	
3	—	—	0.1	—	Dead		
4	—	—	0.1	—	Dead		
5	0.1	—	0.2	—	.....	.....	Survived
6	0.1	—	0.5	—	.....	.....	Survived
7	—	—	—	0.05	.....	.....	Survived
8	—	—	—	0.1	Sick	Dead	
9	—	—	—	0.2	Dead		
10	0.1	—	—	0.1	Dead		
11	0.1	—	—	0.2	Dead		
12	..	0.1	0.1	—	Dead		
13	..	0.1	0.2	..	Dead		
14	..	0.1	0.5	..	Dead		

Table 5 shows that 0.1 c c antiblackleg serum protects against 25 lethal doses of B. chauvœi. On the other hand, a like amount fails entirely to protect against even one fatal dose of Vibrion septique. This method is of great advantage in determining the purity of questionable cultures, and in isolating the Vibrion septique from B. chauvœi, both in cultures and in infected muscle, in case of coexistence of these two species in the same tube or the same muscle, as shown in table 6.

Guinea-pigs weighing 350 gm. were injected with antiblackleg serum, anti-Vibrion septique serum (Pasteur), normal horse serum and a mixture of antiblackleg and anti-Vibrion septique serums. The guinea-pigs were inoculated subcutaneously 24 hours later with 0.2 c c of

20-hour Hibler cultures of B. chauvœi (7) and Vibrion septique (Pasteur). The results show that antiblackleg serum and anti-Vibrion septique serum give specific protection against B. chauvœi and Vibrion septique respectively. The guinea-pigs receiving normal horse serum died from a mixed infection while those receiving both antiblackleg and anti-Vibrion septique serums survived.

Guinea-pigs 1, 2, 3 and 4 presented typical pictures of Vibrion septique, chains and filaments on the liver. Pure cultures of Vibrion septique were obtained from the heart blood in each case.

Guinea-pigs 5 and 6 presented blackleg pictures—no chains or filaments on the surface of the liver. Pure cultures of B. chauvœi were obtained from the heart blood in each case.

The lesions in guinea-pigs 7 and 8 resembled blackleg lesions, but long chains and filaments were found on the surface of the liver. Liver agar cultures from the blood of these animals showed the presence of B. chauvœi and Vibrion septique.

TABLE 6

Antiblackleg and Anti-Vibrion Septique Serums

Guinea-Pig	Serum			B. chauvœi	Vibrion Septique	Results	
	Anti-blackleg	Anti-Vibrion Septique (Pasteur)	Normal Horse			24 Hours	96 Hours
1	0.1	.....	.....	0.2	0.2	Dead	
2	0.1	.....	.....	0.2	0.2	Dead	
3	0.2	.....	.....	0.2	0.2	Dead	
4	0.2	.....	.....	0.2	0.2	Dead	
5	.....	0.4	.....	0.2	0.2	Dead	
6	.....	0.5	.....	0.2	0.2	Dead	
7	.....	.....	0.3	0.2	0.2	Dead	
8	.....	.....	0.5	0.2	0.2	Dead	
9	0.2	0.2	.....	0.2	0.2	Survived	Survived
10	0.2	0.4	.....	0.2	0.2	Survived	Survived

AGGLUTINATION REACTIONS

Statements as to the value of agglutination reactions in the differentiation of anaerobes are conflicting. Many authors find them to indicate close relationship between the various species, and so to be valueless in their separation. Others, particularly MacIntosh and Fildes,[8] state that the agglutination reaction is of great value in separating B. chauvœi and Vibrion septique.

The first difficulty to be overcome in such a study is the rapid sedimentation of B. chauvœi cultures. Believing this flocculability to be caused by electrolytes, such cultures were subjected to repeated washing with distilled water, and were finally suspended in this medium.

[8] Brit. Med. Research Committee Bull. 12.

TABLE 7

AGGLUTINATION OF B. CHAUVŒI AND VIBRION SEPTIQUE BY NORMAL HORSE SERUM

Normal Horse Serum, 0.5 C c	Suspension Vibrion septique	Final Dilution	Agglutination
1:10	0.5 c c	1:20	+++
1:20	0.5 c c	1:40	+++
1:40	0.5 c c	1:80	+++
1:80	0.5 c c	1:160	+++
1:100	0.5 c c	1:200	++
1:200	0.5 c c	1:400	++
1:400	0.5 c c	1:800	++
1:800	0.5 c c	1:1600	—
1:1000	0.5 c c	1:2000	—
		Control	—
	Suspension B. chauvœi		
1:10	0.5 c c	1:20	++
1:20	0.5 c c	1:40	++
1:40	0.5 c c	1:80	++
1:80	0.5 c c	1:160	++
1:100	0.5 c c	1:200	++
1:200	0.5 c c	1:400	—
1:400	0.5 c c	1:800	—
1:800	0.5 c c	1:1600	—
1:1000	0.5 c c	1:2000	—
		Control	—

+++ = complete agglutination; ++ = incomplete agglutination; + = no agglutination.

TABLE 8

AGGLUTINATION OF B. CHAUVŒI AND VIBRION SEPTIQUE BY ANTIBLACKLEG SERUM

Antiblackleg Serum, 0.5 C c	Suspension B. chauvœi	Final Dilution	Agglutination
1:10	0.5 c c	1:20	+++
1:20	0.5 c c	1:40	+++
1:40	0.5 c c	1:80	+++
1:80	0.5 c c	1:160	+++
1:100	0.5 c c	1:200	+++
1:200	0.5 c c	1:400	+++
1:400	0.5 c c	1:800	+++
1:800	0.5 c c	1:1600	—
1:1000	0.5 c c	1:2000	—
		Control	—
	Suspension Vibrion septique		
1:10	0.5 c c	1:20	++
1:20	0.5 c c	1:40	++
1:40	0.5 c c	1:80	++
1:80	0.5 c c	1:160	++
1:100	0.5 c c	1:200	++
1:200	0.5 c c	1:400	—
1:400	0.5 c c	1:800	—
1:800	0.5 c c	1:1600	—
1:1000	0.5 c c	1:2000	—
		Control	—

Fermentation tubes of liver broth were inoculated with B. chauvœi cultures incubated 18 hours at 37 C. and centrifuged. The organisms were suspended in distilled water and washed 5 times, and finally suspended in a volume of distilled water equal to one-half the original culture. Purified cresol, 0.2%, was used as a preservative. This suspension was allowed to stand at room temperature for at least 48 hours. The supernatant only was used when sedimentation took place. Suspensions of Vibrion septique were prepared in the same manner. Suspensions of the same opacity were used.

Such suspensions are perfectly stable and serve very well in agglutination tests. Normal horse serum and antiblackleg serum were tested against suspensions of Vibrion septique and B. chauvœi.

It was found that normal horse serum agglutinates both B. chauvœi and Vibrion septique, the former in titer 1:200, the latter in 1:400. Horse antichauvœi serum agglutinates B. chauvœi in titer 1:800 and Vibrion septique in 1:200. It would appear, therefore, that there is a distinct zone of specificity in these reactions, despite the rather high agglutination titer of the normal horse serum, especially for Vibrion septique. To resolve this question it will be necessary to titer the serum of a large number of normal horses against both of these organisms. The titers obtained should be compared with an equally large number of those from blackleg immune horses. If a satisfactory zone of specificity is found to exist, these tests should be supplemented by careful absorption reactions. For the rest, it must be admitted that this reaction is distinctly in the experimental stage and requires much further elaboration and study.

SUMMARY

The methods of preparation of mediums suitable for the growth of B. chauvœi are described.

B. chauvœi is discussed with regard to cultural and morphologic characteristics.

A method for the rapid isolation of B. chauvœi from infected material is given.

The failure of pure cultures of B. chauvœi to grow on 2% dextrose agar is an important and much neglected criterion for judging the purity of such cultures.

Cultures of B. chauvœi of high virulence for guinea-pigs are fatal to mice, though in much higher doses than are necessary to kill guinea-pigs.

The amount of B. chauvœi culture required to kill a pigeon is many times greater than that required to kill a guinea-pig.

Sheep are apparently somewhat refractory to natural infection with B. chauvœi but can be successfully infected with large doses of virulent cultures. No strain of genuine B. chauvœi has been isolated from the tissues of sheep suspected of having died of blackleg.

Kids may be fatally infected with pure cultures of B. chauvœi.

Protection tests with antiblackleg serum indicate a marked specific for B. chauvœi, and assist materially in its identification and its differentiation from Vibrion septique.

It is possible that the agglutination reaction may be of use in the identification of this organism, but its study is still distinctly in the experimental stage.